SUBSTITUTE FORM PTO-1390

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 07898/038001

U.S. APPLICATION NO (IF KNOWN): NOT KNOWN 09/284114

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP97/03591	October 7, 1997	October 8, 1996

TITLE OF INVENTION
A MOUSE STRAIN WITH NATURAL ONSET OF AUTOIMMUNE ARTHRITIS

APPLICANT(S) FOR DO/EO/US
SHIMON SAKAGUCHI

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ■ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ■ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ■ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ■ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ■ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ■ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ■ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ■ have not been made and will not be made.

8. ☐ A translation of amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ■ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other documents or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ■ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☐ Other items or information:

"Express Mail" mailing label number EL253175391US
Date of Deposit 07 APRIL 1999
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231

M. E. Augustine
M. E. Augustine

U.S. APPLICATION NO. (IF KNOWN)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
	PCT/JP97/03591	07898/038001

17. ■ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search report has been prepared by the EPO or JPO .... $ 840 International preliminary examination fee paid to USPTO (37 CFR 1.482).... $ 670 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))........... $ 760 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO............... $ 970 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2) to (4)........... $ 96		
ENTER APPROPRIATE BASIC FEE AMOUNT	**$ 840.00**	
Surcharge of $130 for furnishing the oath or declaration later than ☐ 20 ☐ 30 mos from the earliest claimed priority date (37 CFR 1.492(e)).	**$ 00.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
TOTAL CLAIMS	1 - 20	00	x $ 18	**$ 00.00**	
INDEPENDENT CLAIMS	1 - 3	00	x $ 78	**$ 00.00**	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270	**$ 00.00**	

TOTAL OF ABOVE CALCULATIONS	**$ 840.00**	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28.)	**$ 00.00**	
SUBTOTAL	**$ 00.00**	
Processing fee of $130 for furnishing the English Translation later than ☐ 20 ☐ 30 mos. from the earliest claimed priority date (37 CFR 1.492(f))	**$ 00.00**	
TOTAL NATIONAL FEE	**$ 840.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31).	**$ 40.00**	
TOTAL FEES ENCLOSED	**$ 880.00**	
	Amount to be refunded	
	Charged	

a. ■ A check in the amount of $880.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 06-1050 in the amount of $ _________ to cover the above fees. A duplicate copy of this sheet is enclosed.
c. ■ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1050. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Lisa A. Haile, Ph.D.
FISH & RICHARDSON P.C.
4225 Executive Square, Suite 1400
La Jolla, California 92037

Lisa A. Haile
SIGNATURE

Lisa A. Haile, Ph.D.
NAME

38,347
REGISTRATION NUMBER

DESCRIPTION

A MOUSE STRAIN WITH NATURAL ONSET OF AUTOIMMUNE ARTHRITIS

TECHNICAL FIELD

The present invention relates to a mouse model with natural onset of morbid conditions strikingly similar to those of rheumatoid arthritis developing in humans. This strain of mice is useful as an animal model of rheumatoid arthritis.

BACKGROUND ART

Among autoimmune diseases, rheumatoid arthritis is the most frequent disease; for example, the number of patients with this disease in the US is estimated to be 6.5 millions. The cause and pathogenetic mechanism of this disease are largely unknown at present.

For elucidation of the cause and mechanism of diseases of unknown etiology, animal models are useful, especially when they naturally develop the diseases clinically and pathologically similar to the human counterparts. For example, the NOD strain of mice develop insulin-dependent diabetes mellitus, which is an autoimmune disease like rheumatoid arthritis (Makino, S. et al. Exp. Animals (Tokyo) 29, 1-13, 1980). NZB and NZW mice are used widely as a model for systemic lupus erythematosus (SLE) (Andrews, B. S. et al., J. Exp. Med. 148, 1198-1215, 1978). These animals have greatly contributed to the elucidation of the cause and mechanism of respective diseases.

Some animals showing similar morbid conditions to those of rheumatoid arthritis in humans are also known. For example, MRL-lpr/lpr mice show natural onset of arthritis mainly in the leg

joints (Hang, L. et al., J. Exp. Med. 155:1690, 1982). However, the arthritis in this strain is generally mild, and the maintenance of the strain for a prolonged period is difficult because of abnormal proliferation of lymphocytes in the lymph nodes and spleen, hampering the wide use of the strain as a model of arthritis. Collagen arthritis can be induced in mice by immunizing with type II collagen, which is abundant in joints, along with strong adjuvant (Stuart, J. M. et al., Annual Rev. Immunol. 2:199, 1984). Adjuvant arthritis can also be induced in rats by immunizing with dead tubercule bacilli (Taurog, J. D. et al., Cell Immunol. 75:271, 1983). Although these models show morbid conditions similar to those of rheumatoid arthritis, the relationship of human rheumatoid arthritis to the abnormality of type II collagen or to the infection with tubercule bacilli is not proven. Accordingly, the findings obtained by utilizing these model animals cannot necessarily be extrapolated to humans, and it is contentions whether these animals can be used as suitable models of human rheumatoid arthritis.

PROBLEM TO BE SOLVED BY THE INVENTION

An animal model with immunopathological characteristics of rheumatoid arthritis, is necessary for development of effective therapies for rheumatoid arthritis in humans. The present invention meets such a requirement, and the object of the invention is to provide a mouse model with natural onset of morbid conditions strikingly similar to those of rheumatoid arthritis in humans.

DISCLOSURE OF THE INVENTION

As a result of his extensive study for solving the problems described above, the present inventor found a mouse with joint swelling among a normal BALB/c colony, and from this finding, attained the present invention. Hence, the present invention is a mouse strain having the character of natural onset of autoimmune arthritis. This mouse strain was designated as the SKG strain.

Hereinafter, the present invention is described in detail.

The mouse of the invention, which was designated as the SKG strain, possesses the character of natural onset of autoimmune arthritis. Although the time of onset of autoimmune arthritis varies among individual mice, the onset of the arthritis is usually about 3 to 4 months after birth. As described in the "BACKGROUND ART" above, MRL-lpr/lpr mice also show natural onset of arthritis. However, the mouse of the invention and the MRL strain are different in their morbid conditions. For example, the arthritis in the MRL strain is generally localized to the joints of the hind legs, and even after progressing chronically, does not lead to joint stiffening, while the arthritis in the mouse of the invention develops in the joints of the forelegs and hind legs, and chronically progressing to joint stiffening. Furthermore, the mouse of the invention does not show the abnormal proliferation of lymphocytes or the SLE-like lesions observed in the MRL strain.

The autoimmune arthritis observed in the mouse of the invention is strikingly similar to human rheumatoid arthritis in morbid conditions. Specifically, there are the following similarities therebetween:

1) It pathohistologically resembles human rheumatoid arthritis in

its chronic progression from the appearance of pannus to the inflammatory destruction of joint cartilage and bone accompanied by lymphocyte infiltration (Figs. 9 and 11).

2) Clinically, it resembles human rheumatoid arthritis in that the small and large joints of the forelegs and hind legs are affected symmetrically, and in that the lesions chronically progress and finally lead to joint stiffening (Figs. 1, 3, 5 and 7).

3) It resembles human rheumatoid arthritis in that rheumatoid factor, autoantibody against type II collagen specific for joints, and hypergammaglobulinemia develops highly frequently in the mouse of invention (Figs. 14, 15 and 16).

From these similarities, the mouse of the invention can be used as a good model of human rheumatoid arthritis.

The mouse of the invention can be produced by mating between SKG strain of mice or by mating them with other suitable strains of mice and selecting the obtained mice for those having the characters described above. The applicant will distribute the SKG strain of mice in accordance with the stipulation of Article 27-3, Item 1, of the Japanese Patent Law Enforcement Regulations.

EXAMPLES

Example 1

In 1993, a female mouse with joint swelling was found in an inventor's BALB/c colony (purchased in 1992 from Nippon SLC) in the Institute for Physical and Chemical Research. This joint swelling was assumed to be due to a genetic mutation; and this mutant strain was designated as SKG. The following experiments were conducted to examine the properties of its gene.

The SKG mouse having developed arthritis was mated with a BALB/c mouse (originally purchased from Nippon SLC). As the result of this mating, 12 mice were obtained, among which 4 mice (3 females and 1 male) showed joint swelling (the incidence of arthritis: 33 %). One mouse was arbitrarily selected from the mice having joint swelling and mated again with a BALB/c mouse in a mouse colony (originally purchased from Nippon SLC) maintained in the inventor's laboratory. As the result of this mating, 15 mice were obtained, among which 6 mice (4 females and 2 males)had joint swelling (the incidence of arthritis: 40 %). One mouse was arbitrarily selected from the mice having joint swelling and mated again with another mouse in the above-described BALB/c colony. As the result, 28 mice were obtained, among which 10 mice had joint swelling (the incidence of arthritis: 35 %). As the result of the above matings through 3 generations, arthritis developed at the incidence of 30 to 40 % in both male and female mice when mated with BALB/c mice in the inventor's colony.

It was initially considered that the BALB/c mice used in the mating experiments described above were normal and had not developed arthritis. It was also considered from the above results that the gene causing the natural onset of the autoimmune arthritis showed autosomal inheritance dominant. However, in later experiments, the BALB/c mice considered normal and apparently free of swelling in large joints (e.g. leg joints), were found by detailed observation for a long period (6 months or more)to have joint swelling in small joints of the fingers. Furthermore, although the incidence of arthritis in large joints was not 100% as described above, the total incidence of arthritis was found to be nearly 100 % if the swelling of small joints was

taken into account. Judging from these results, the type of inheritance of the arthritis was considered to be incompletely dominant or recessive. By later experiments on the inheritance in a large scale, it was reasonably estimated that the genetic abnormality causing the natural onset of autoimmune arthritis is autosomal and recessive. The SKG mice are therefore maintained at present as homozygotes. Their incidence of arthritis is almost 100 %, and the penetrance of the genetic abnormality in the homozygotes is considered to be almost 100 % in the environment where they are currently maintained.

Example 2

The forelegs and hind legs of a SKG mouse (6-month-old) having developed arthritis were observed with the naked eye. A photograph of a foreleg is shown in Fig. 1; and a photograph of a hind leg is shown in Fig. 3. As the control, photographs of a foreleg and hind leg of a normal mouse are shown in Figs. 2 and 4, respectively.

As shown in Figs. 1 and 3, swelling is observed in the joints of the forelegs and hind legs of the mouse having developed arthritis.

Example 3

X-ray photographs were taken of forelegs and hind legs of a SKG mouse (6-month-old) having developed arthritis. The photograph of the forelegs is shown in Fig. 5 and the photograph of the hind legs is shown in Fig. 7. As controls, photographs of forelegs and hind legs of a normal BACB/c mouse of the same age are shown in Figs. 6 and 8, respectively.

As shown in Figs. 5 and 7, the cartilage and bone are destroyed symmetrically in the large and small joints of the

foreleg and hind leg.

Example 4

The joint of the hind leg of a SKG mouse (5-month-old) having developed arthritis was fixed in 10 % formalin for 3 days, embedded in paraffin, cut into a thin section and stained with hematoxylin-eosin. A similar section was prepared from a normal mouse and stained.

A microscopic photograph of a section of joint tissues from a SKG mouse having developed arthritis is shown in Fig. 9 (magnification: X 40) and Fig. 11 (magnification: X 400). A microscopic photograph of a similar section from a normal mouse is shown in Fig. 10 (magnification: X 40) and Fig. 12 (magnification: X 400).

Fig. 9 shows disappearance of the articular cavity, destruction of the cartilage and bone, and infiltration of inflammatory cells. Fig. 11 with further magnification indicates pannus formation, infiltration of inflammatory cells, and destruction of joint cartilage and bone.

Example 5

SKG mice (5- to 6-month-old) and normal BALB/c mice (5- to 6-month-old) were examined for the thickness of the left ankle joint. Fifteen mice each were examined. The result is shown in Fig. 13.

As shown in Fig. 13, the mice having developed arthritis had increased diameters of the ankle joint, as compared with those of the normal mice.

Example 6

SKG mice (5- to 6-month-old) and normal BALB/c mice (5- to 6-month-old) were examined by ELISA for the titer of IgM antibody

(rheumatoid factor) against mouse immunoglobulin G (IgG). Fifteen animals each were examined. The result is shown in Fig. 14.

As shown in Fig. 14, the SKG mice having developed arthritis had significantly increased titers of rheumatoid factor, as compared with those of the normal BALB/c mice.

Example 7

SKG mice (5- to 6-month-old) and normal BALB/c mice (5- to 6-month-old) were examined by ELISA for the titer of circulating antibody against bovine type II collagen. Fifteen animals each were examined. The result is shown in Fig. 15.

As shown in Fig. 15, high titers of the autoantibody appear in the SKG mice with arthritis.

Example 8

SKG mice (5- to 6-month-old) and normal BALB/c mice (5- to 6-month-old) were examined for serum IgG levels by SRID (single radial immunodiffusion). Fifteen animals each were examined. The result is shown in Fig. 16.

As shown in Fig. 16, hypergammaglobulinemia is observed in the SKG mice having developed arthritis.

Example 9

Cell suspensions prepared from spleen and lymph node cells of the mice having developed arthritis were cultured in vitro for 3 days in the presence of concanavalin A, and the resulting 3 X 10^7 activated T cells were transferred intravenously to normal BALB/c nude mice (6-week-old). Two months after transfer, all the nude mice (7 animals) to which the cells had been transferred showed swelling of the joints of the hind legs. After 3 months, histological sections prepared as in Example 4 showed

histological characteristics similar to those in Fig. 9 and 11.

EFFECT OF THE INVENTION

The present invention relates to a mouse model with natural onset of the morbid conditions strikingly similar to those of human rheumatoid arthritis. This mouse is useful as an animal model of rheumatoid arthritis.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a photograph of a foreleg of a SKG mouse having developed arthritis.

Fig. 2 is a photograph of a foreleg of a normal BALB/c mouse.

Fig. 3 is a photograph of a hind leg of a SKG mouse having developed arthritis.

Fig. 4 is a photograph of a hind leg of a normal BALB/c mouse.

Fig. 5 is an X-ray photograph of forelegs of a SKG mouse having developed arthritis.

Fig. 6 is an X-ray photograph of forelegs of a normal BALB/c mouse.

Fig. 7 is an X-ray photograph of hind legs of a SKG mouse having developed arthritis.

Fig. 8 is an X-ray photograph of hind legs of a normal BALB/c mouse.

Fig. 9 is a microscopic photograph (magnification: X 40) of a section of the joint tissue prepared from a SKG mouse having developed arthritis.

Fig. 10 is a microscopic photograph (magnification: X 40)

of a section of the joint tissue prepared from a normal BALB/c mouse.

Fig. 11 is a microscopic photograph (magnification: X 400) of a section of the joint tissue prepared from a SKG mouse having developed arthritis.

Fig. 12 is a microscopic photograph (magnification: X 400) of a section of the joint tissue prepared from a normal BALB/c mouse.

Fig. 13 is a graph showing the thickness of the left ankle joints of SKG mice at 5~6 months of age.

Fig. 14 is a graph showing the titer of rheumatoid factor in SKG mice at 5~6 months of age.

Fig. 15 is a graph showing the titer of autoantibody against type II collagen in SKG mice at 5~6 months of age.

Fig. 16 is a graph showing serum IgG levels in SKG mice at 5~6 months of age.

CLAIM

1. A mouse strain having the character of natural onset of autoimmune arthritis, the character being derived from the SKG strain.

ABSTRACT

The present invention provides a mouse model with natural onset of morbid conditions strikingly similar to those of rheumatoid arthritis in humans. This mouse strain can be utilized as an animal model of rheumatoid arthritis.

FIG. 1

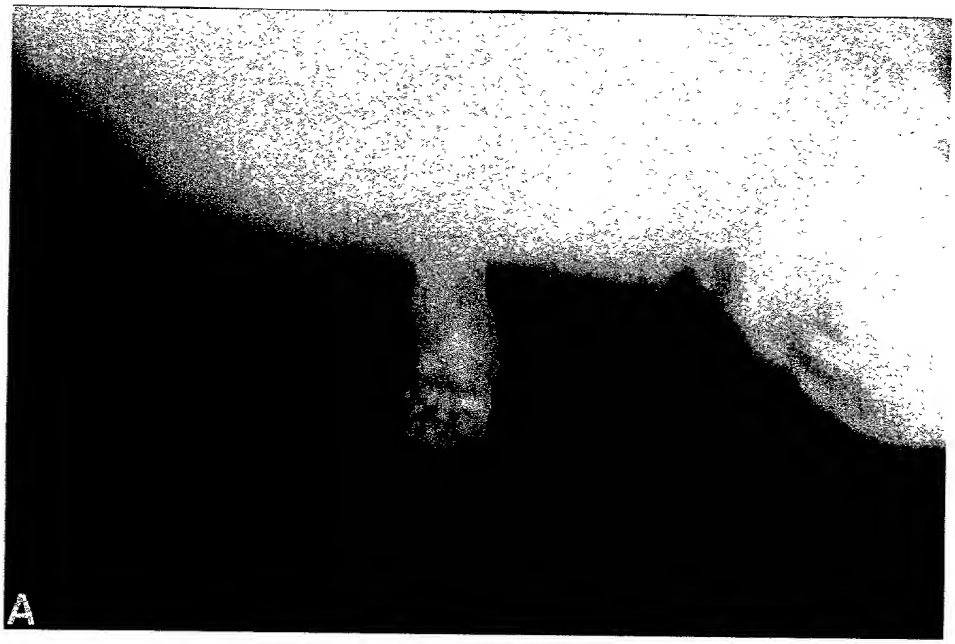


FIG. 2

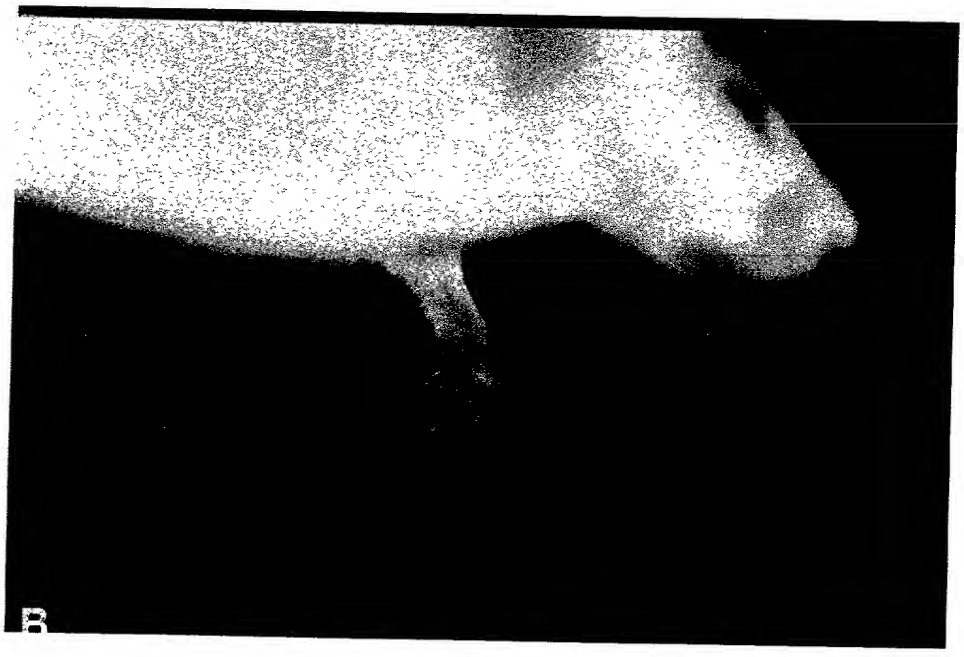


FIG. 3

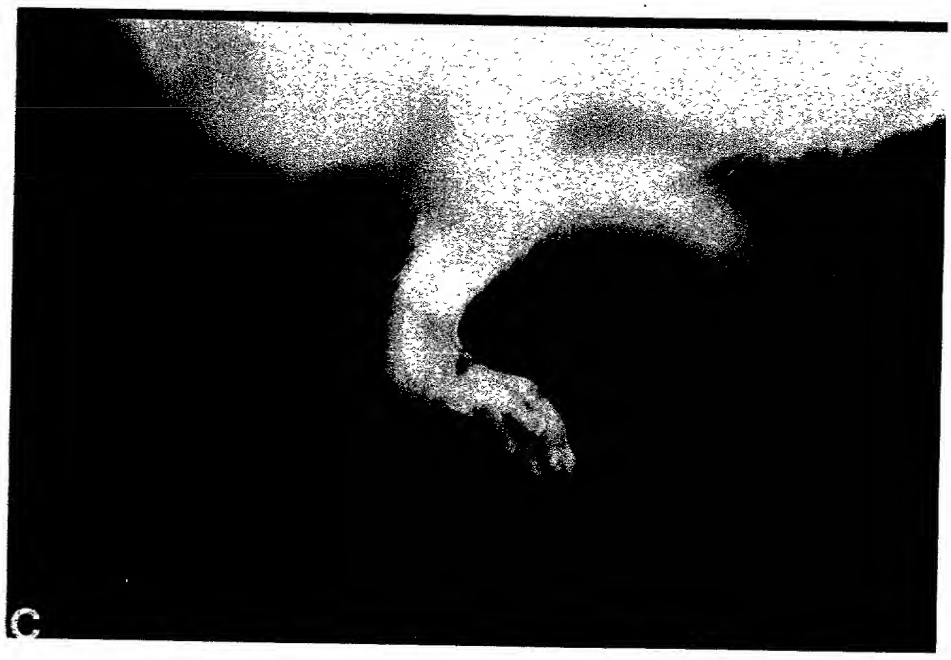


FIG. 4

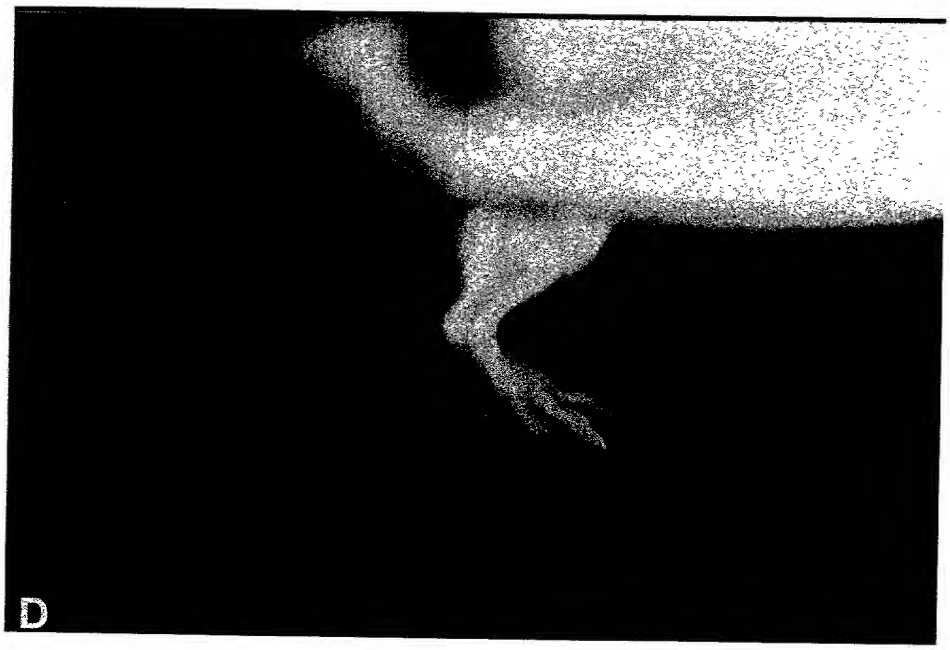


FIG. 5

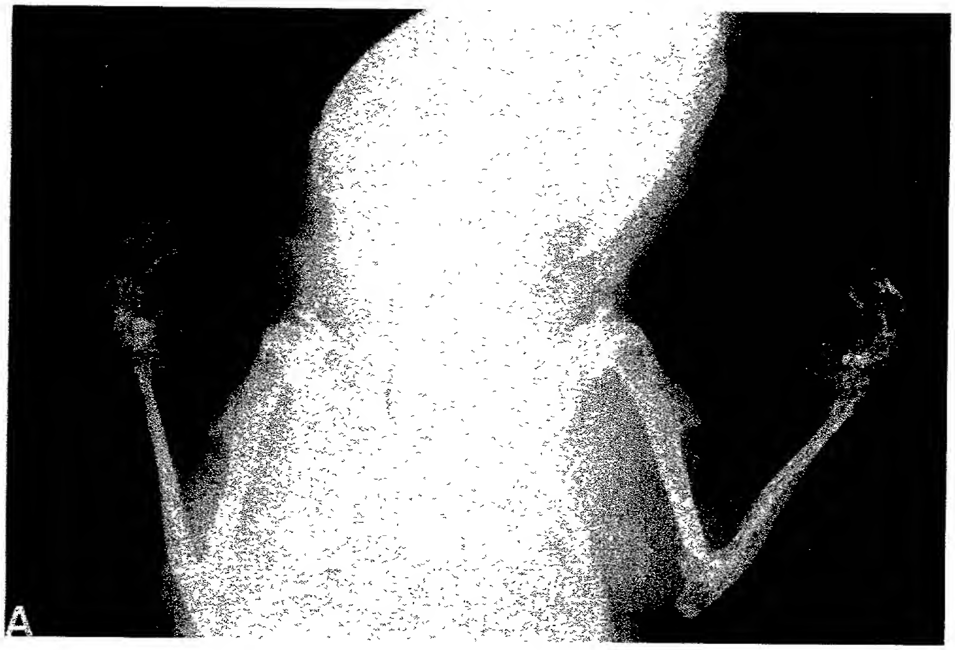


FIG. 6

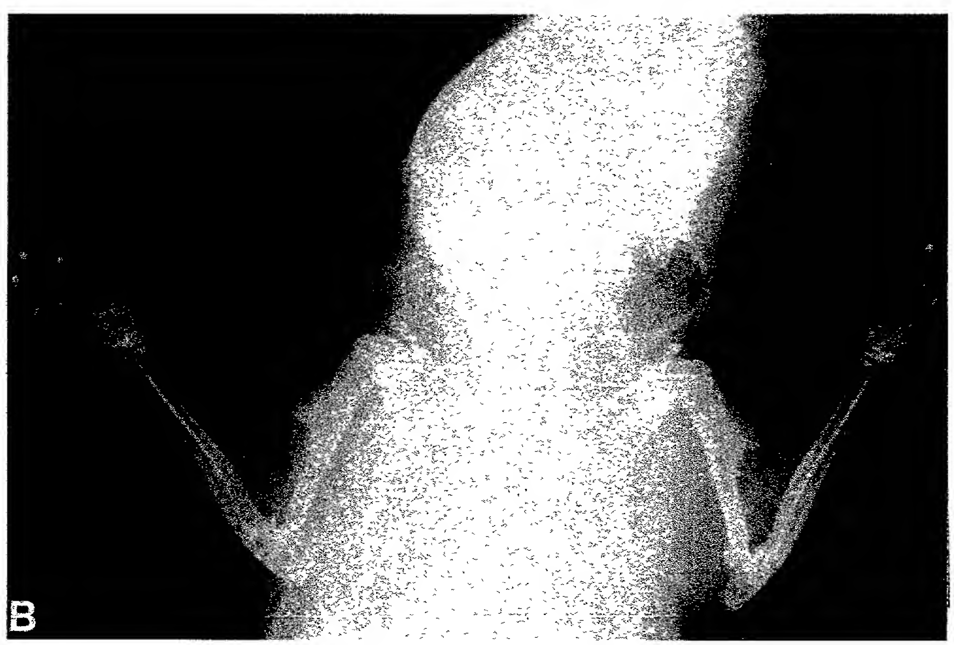


FIG. 7

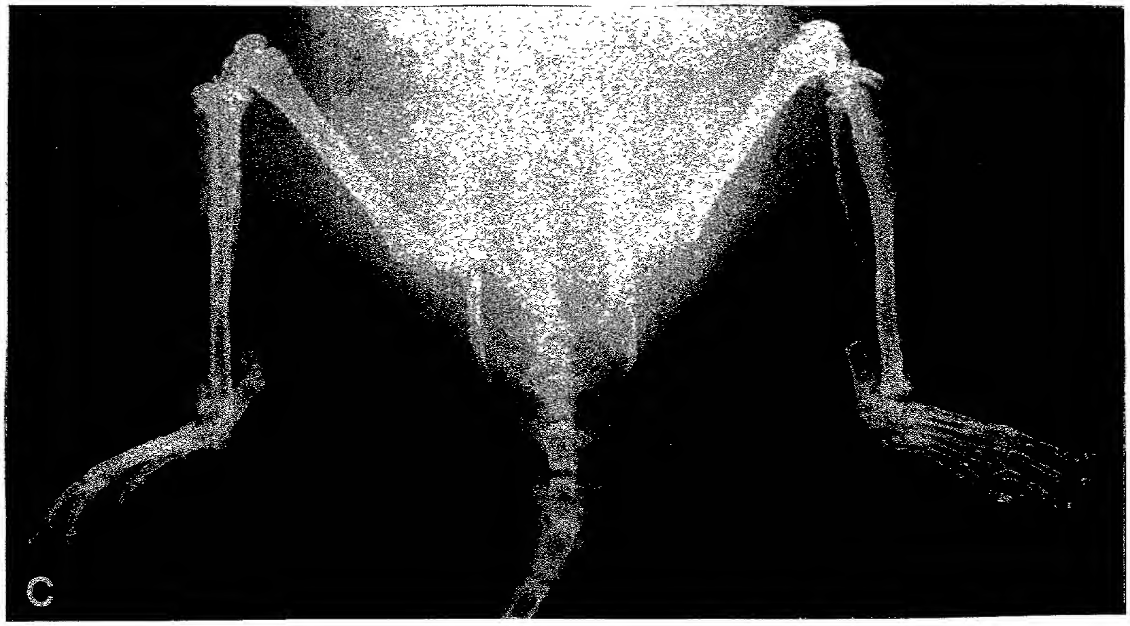


FIG. 8

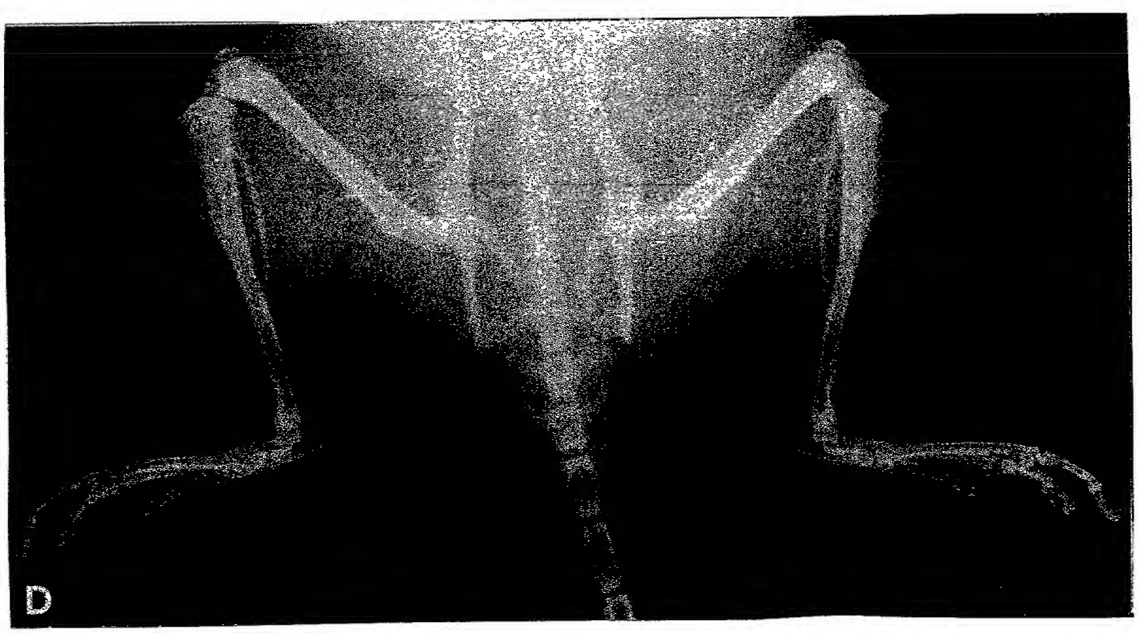


FIG. 9

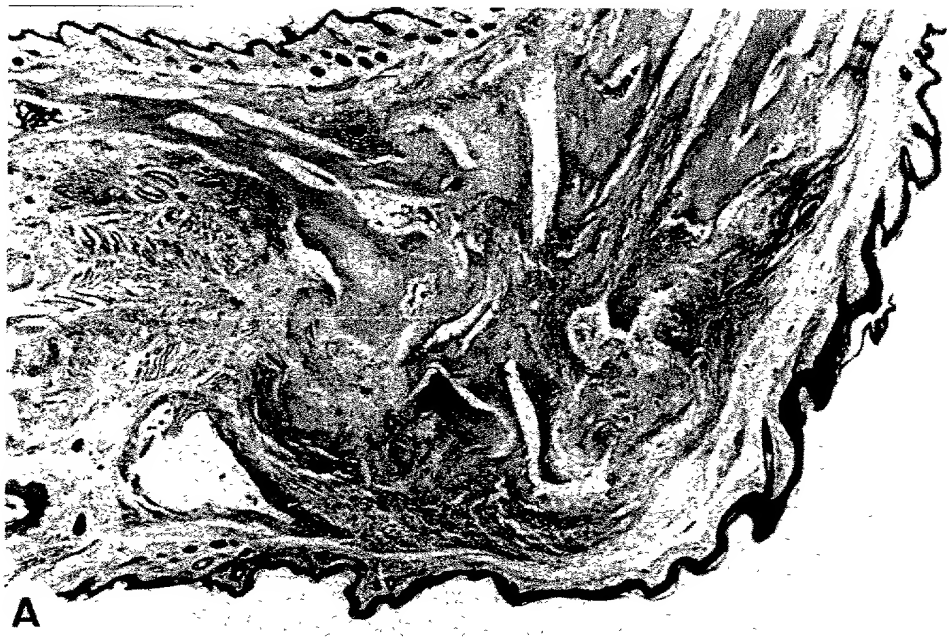


FIG. 10

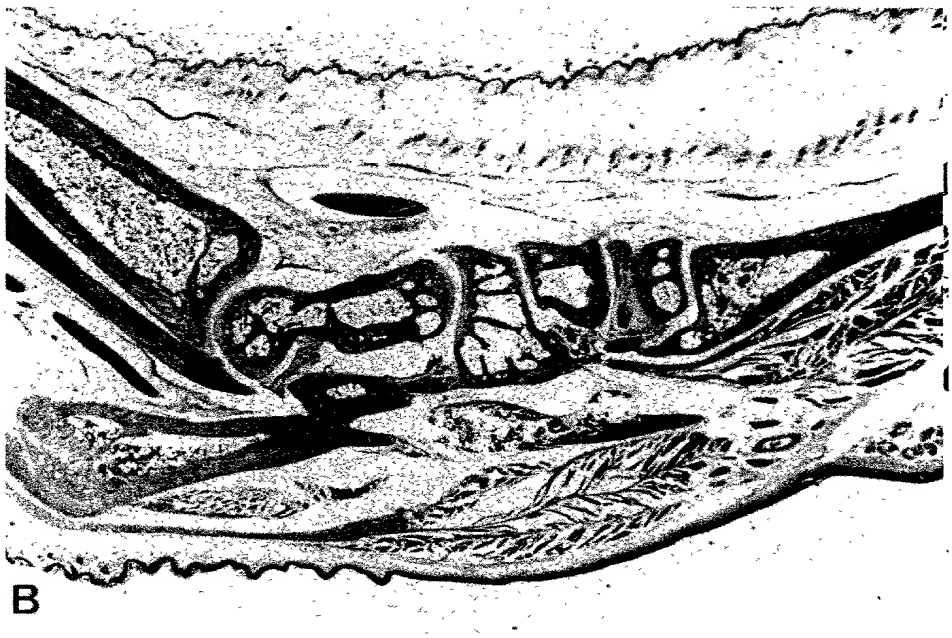


FIG. 11

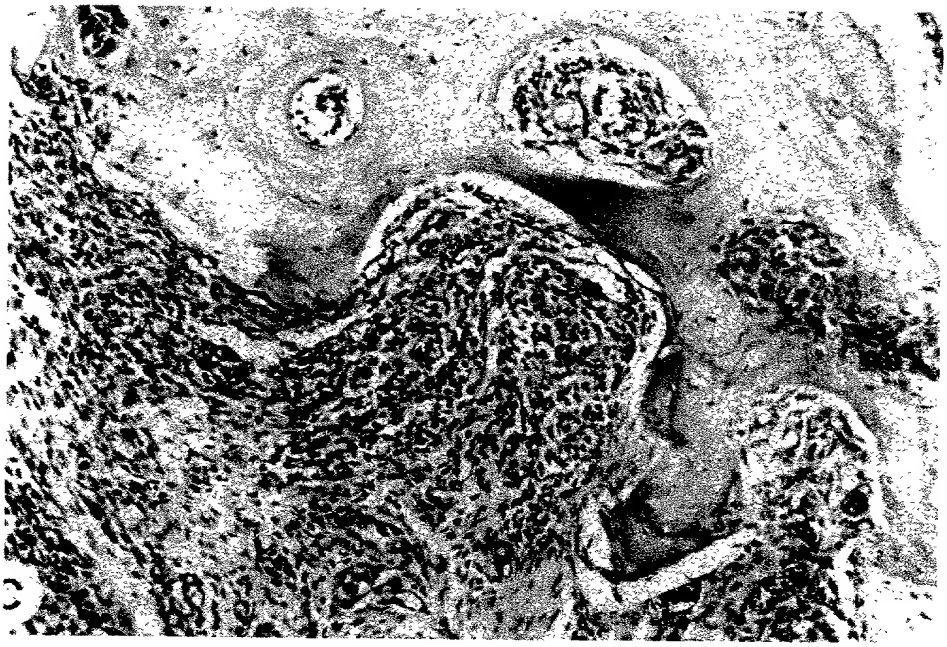


FIG. 12

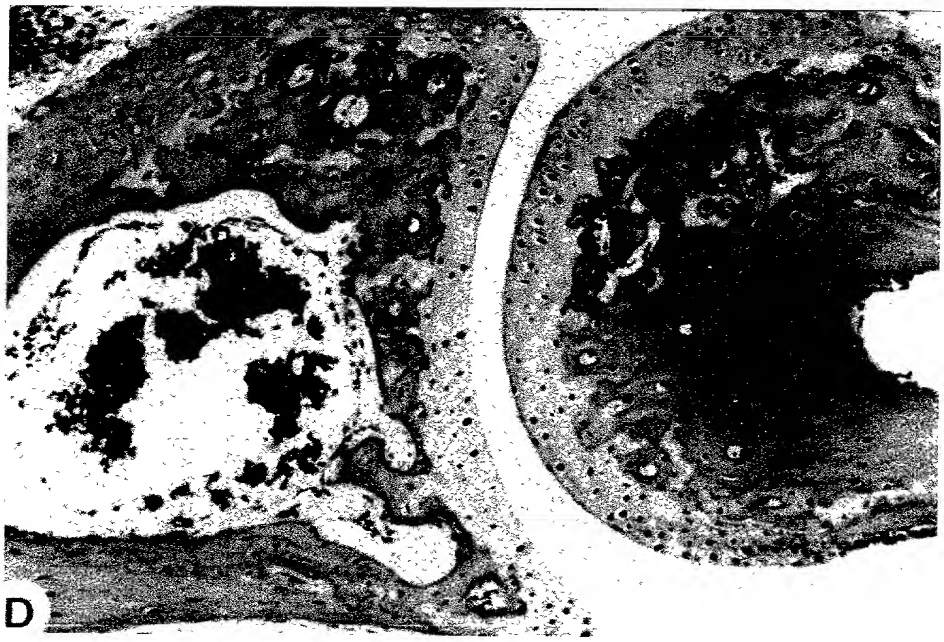


FIG. 13

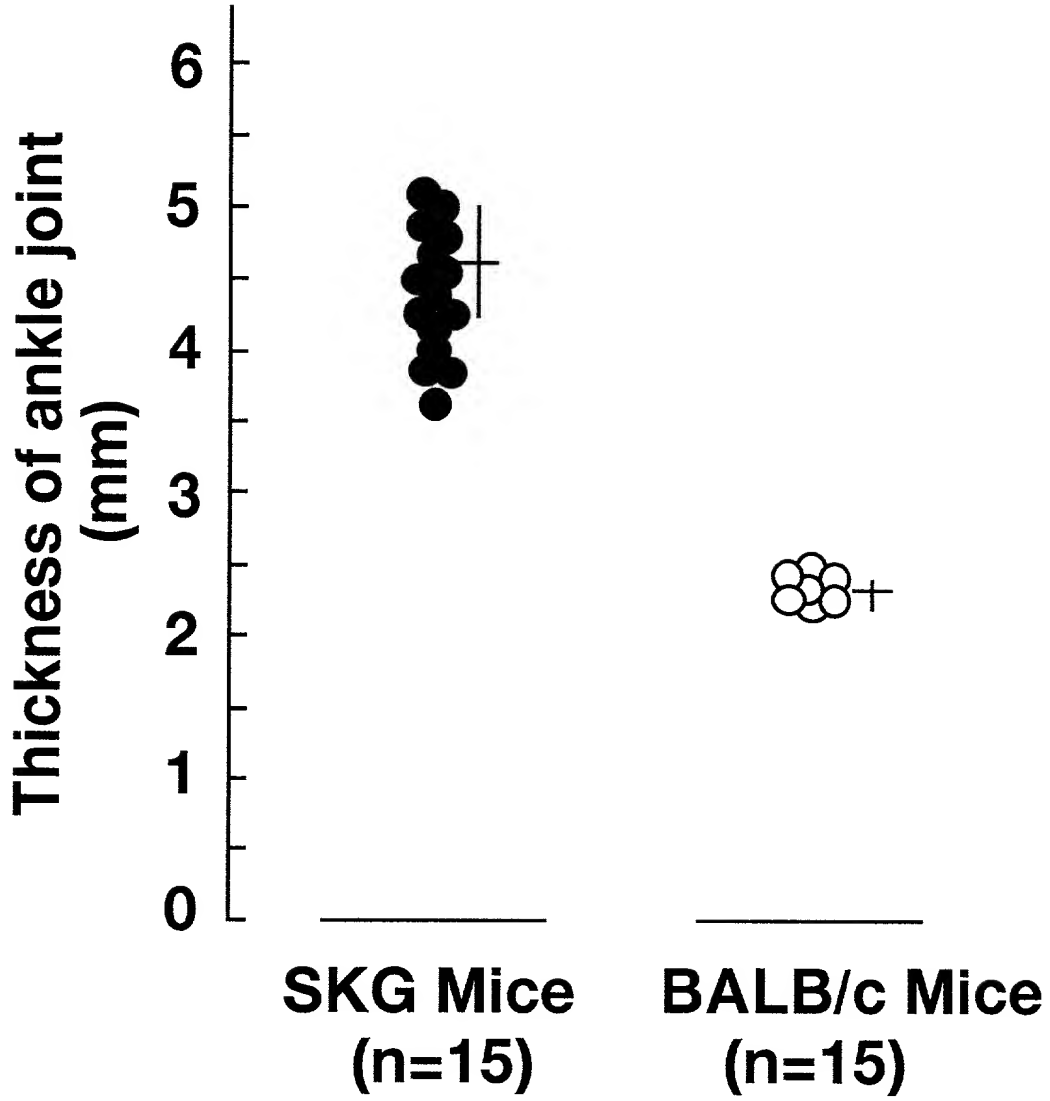

Thickness of ankle joint (mm)
6
5
4
3
2
1
0
SKG Mice (n=15)
BALB/c Mice (n=15)

FIG. 14

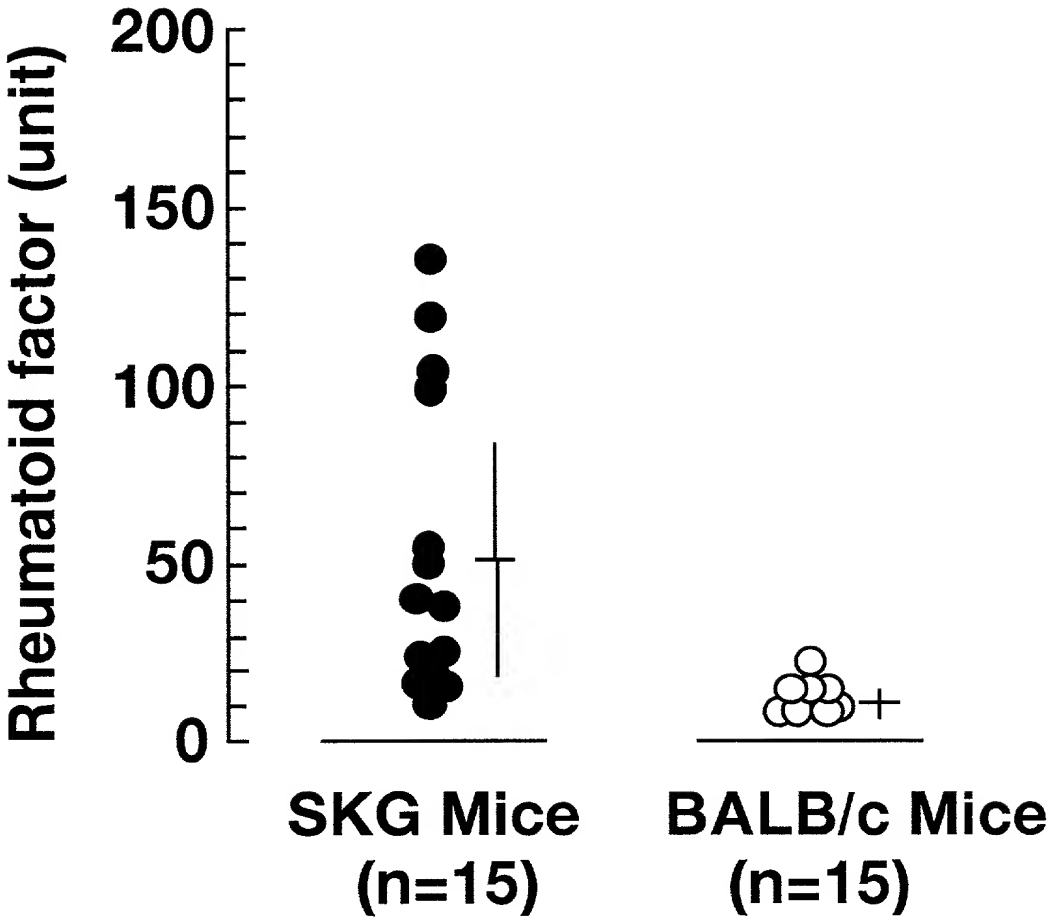

Rheumatoid factor (unit)
200
150
100
50
0
SKG Mice
(n=15)
BALB/c Mice
(n=15)

FIG. 15

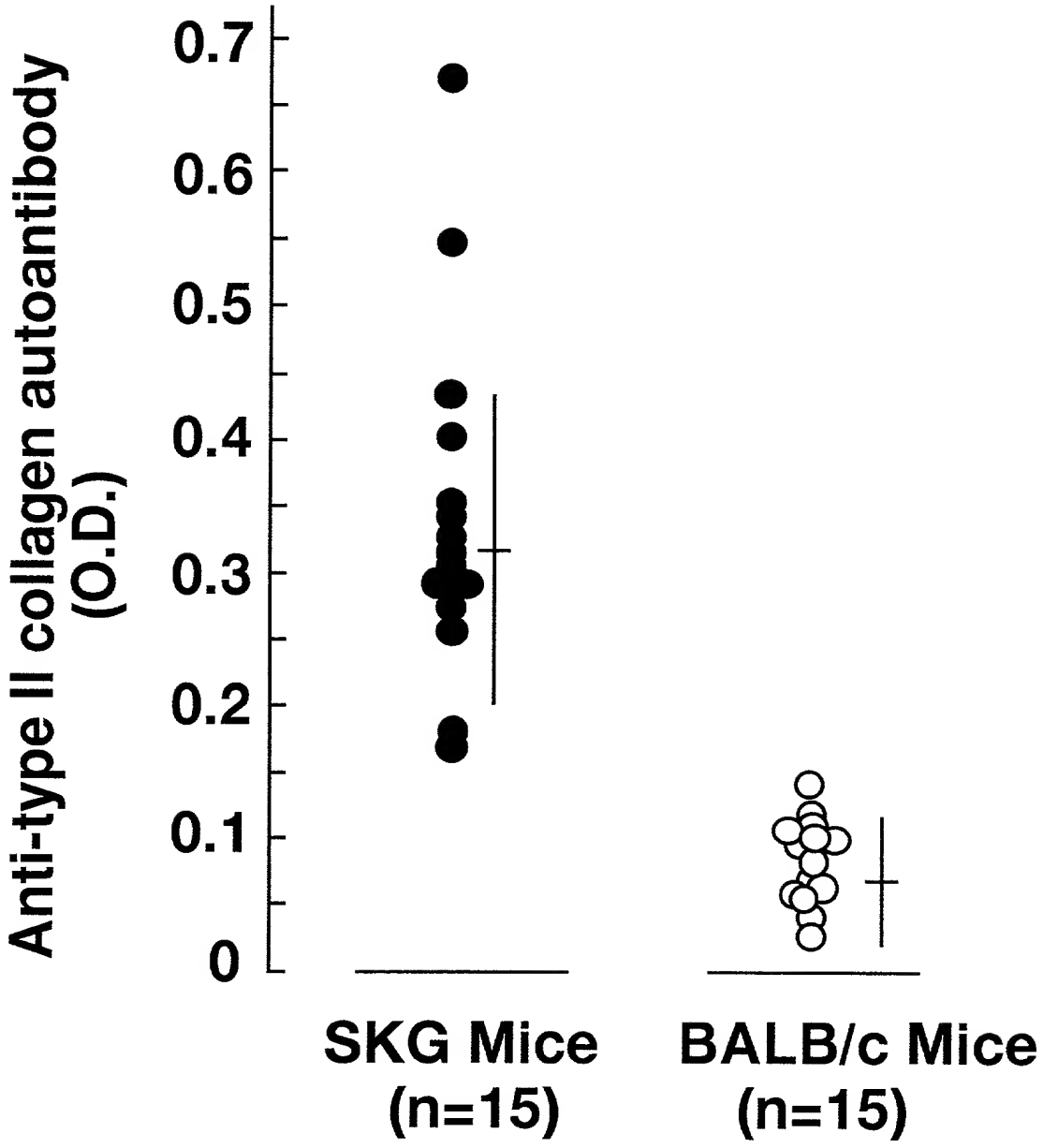
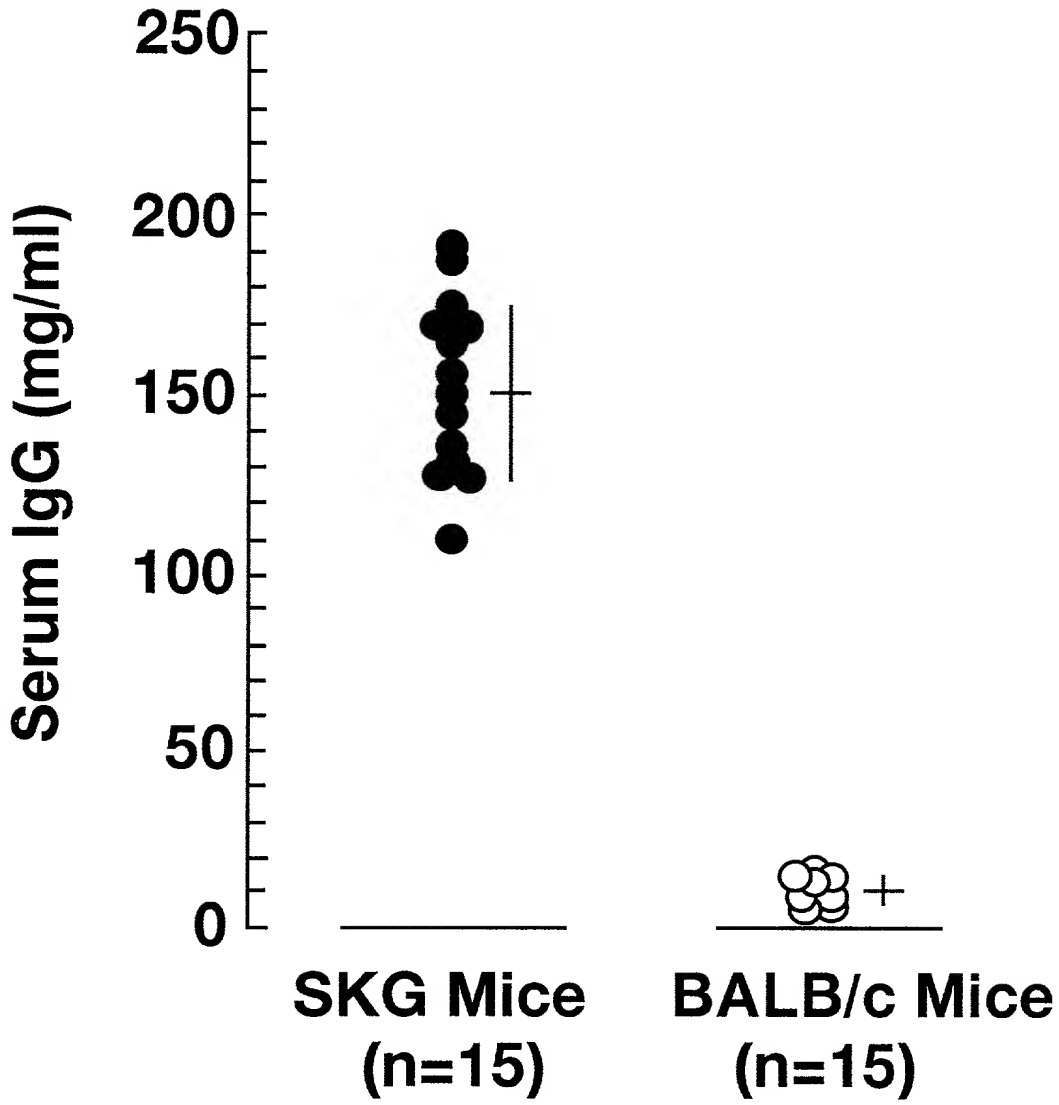

Anti-type II collagen autoantibody (O.D.)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
SKG Mice (n=15)
BALB/c Mice (n=15)

FIG. 16



Attorney's Docket No.: 07898/038001

DECLARATION, POWER OF ATTORNEY AND PETITION

I (We), the undersigned inventor(s), hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I (We) believe that I am (we are) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

A MOUSE STRAIN WITH NATURAL ONSET OF AUTOIMMUNE ARTHRITIS

the specification of which

☐ is attached hereto.

☐ was filed on _____ as Application Serial No. _____ and amended on _____.

■ was filed as PCT international application Number PCT/JP97/03591 on October 7, 1997, and was amended under PCT Article 19 on _____ (if applicable).

I (We) hereby state that I (We) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above; that I (We) do not know and do not believe that this invention was ever known or used before my invention or discovery thereof, or patented or described in any printed publication in any country before my invention or discovery thereof, or more than one year prior to this application, or in public use or on sale in the United States for more than one year prior to this application; that this invention or discovery has not been patented or made the subject of an inventor's certificate in any country foreign to the United States on an application filed by me or my legal representatives or assigns more than twelve months before this application.

I (We) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

I (We) hereby claim foreign priority benefits under Section 119(a)-(d) of Title 35 United States Code, of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Application No.	Country	Filing date	Priority claimed	
267126/1996	Japan	October 8, 1996	■ Yes	☐ No
JP97/03591	WO	October 8, 1997	☒ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No

I hereby claim the benefit under Section 119(e) of Title 35 United States Code, of any United States application(s) listed below.

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

I (We) hereby claim the benefit under Section 120 of Title 35 United States Code, of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Section 112 of Title 35 United States Code, I (We) acknowledge the duty to disclose material information as defined in Section 1.56(a) of Title 37 Code of Federal Regulations, which occurred between the filing date of the prior application and national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status (pending, patented, abandoned)

And I (We) hereby appoint: William E. Booth, Registration No. 28,933; Margaret A. Boulware, Registration No. 28,708; Karl Bozicevic, Registration No. 28,807; Barry E. Bretschneider, Registration No. 28,055; Paul T. Clark, Registration No. 30,162; Peter J. Devlin, Registration No. 31,753; William J. Egan, Registration No. 28,411; Willis M. Ertman, Registration No. 18,658; David L. Feigenbaum, Registration No. 30,378; Janis K. Fraser, Registration No. 34,819; John W. Freeman, Registration No. 29,066; Timothy A. French, Registration No. 30,175; Alan H. Gordon, Registration No. 26,168; Scott C. Harris, Registration No. 32,030; Mark J. Hebert, Registration No. 31,766; Gilbert H. Hennessey, Registration No. 25,759; Charles Hieken, Registration No. 18,411; Robert E. Hillman, Registration No. 22,837; John F. Land, Registration No. 29,554; G. Roger Lee, Registration No. 28,963; Steven E. Lipman, Registration No. 30,011; Gregory A. Madera, Registration No. 28,878; Ralph A. Mittelberger, Registration No. 33,195; Ronald E. Myrick, Registration No. 26,315; Robert C. Nabinger, Registration No. 33,431; Frank P. Porcelli, Registration No. 27,374; Eric L. Prahl, Registration No. 32,590; Alan D. Rosenthal, Registration No. 27,833; Richard M. Sharkansky, Registration No. 25,800; John M. Skenyon, Registration No. 27,468; Michael O. Sutton, Registration No. 26,675; Reginald J. Suyat, Registration No. 28,172; Rene D. Tegtmeyer, Registration No. 33,567; Hans R. Troesch, Registration No. 36,950; John R. Wetherell, Registration No. 31,678; Wayne E. Willenberg, Registration No. 28,488; John N. Williams, Registration No. 18,948; Gary A. Walpert, Registration No. 26,098; Dorothy P. Whelan, Registration No. 33,814; and Charles C. Winchester, Registration No. 21,040; Lisa A. Haile, Registration No. 38,347; John R. Wetherell, Jr., Registration No. 31,678; John W. Freeman, Registration No. 29,066; Scott C. Harris, Registration No. 32,030; John F. Land, Registration No. 29,554; and Hans R. Troesch, Registration No. 36,950.

I(We) hereby request that all correspondence regarding this application be sent to the firm of FISH & RICHARDSON P.C. whose Post office address is: 4225 Executive Square, Suite 1400, La Jolla, California 92037 U.S.A.

I (We) declare further that all statements made herein of my (our) knowledge are true and that all statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Shimon SAKAGUCHI

NAME OF FIRST SOLE INVENTOR

1-00

Shimon Sakaguchi

Signature of Inventor

March 29, 1999

Date

Residence: Tokyo, Japan

Citizen of: Japan

Post Office Address: 1-1-1-603,
Toshinncho, Itabashi-ku,
~~Tokyo~~ 174-0074 Japan

JPY